

What is claimed is:

- 1 1. An isolated hyperactive reverse transcriptase comprising one or more point mutations in
2 the processivity domain and one or more point mutations in the nucleotide selection domain.
- 1 2. The reverse transcriptase of claim 1, wherein the reverse transcriptase is selected from the
2 group consisting of AMV, M-MLV, HTLV-1, BLV, RSV, HFV, R2 *Bombyx mori*, and HIV
3 reverse transcriptase.
- 1 3. The reverse transcriptase of claim 1, wherein the reverse transcriptase is encoded by a
2 modified nucleotide sequence that encodes a modified amino acid sequence modified in the
3 processivity domain corresponding to amino acids 497 to 671 of M-MLV reverse transcriptase.
- 1 4. The reverse transcriptase of claim 1, wherein the reverse transcriptase is encoded by a
2 modified sequence that encodes a modified amino acid sequence modified in the nucleotide
3 selection domain corresponding to amino acids 153 to 158 of M-MLV reverse transcriptase.
- 1 5. The reverse transcriptase of claim 1, wherein the reverse transcriptase may be used in the
2 preparation of full-length cDNA.
- 1 6. The reverse transcriptase of claim 1, wherein the reverse transcriptase comprises reverse
2 transcriptase produced recombinantly.
- 1 7. The reverse transcriptase of claim 1, wherein the reverse transcriptase is purified.
- 1 8. The reverse transcriptase of claim 1, wherein the reverse transcriptase is purified and is
2 greater than 90% pure.
- 1 9. The reverse transcriptase of claim 1, wherein the mutation in the processivity domain
2 comprises one or more of mutations in the following residues in MMLV-RT: H638, Y586, D653,
3 D524, D524 and E562.
- 1 10. The reverse transcriptase of claim 1, wherein the mutation in the processivity domain
2 comprises one or more of the following mutations corresponding to the amino acids in MMLV-
3 RT: H638G, Y586A, D653N, D524N, D524E and E562D.

- 1 11. The reverse transcriptase of claim 1, wherein the mutation in the nucleotide selection
2 domain comprises one or more mutations in the following residues in MMLV-RT: F155, D153,
3 A154, F155, F156, C157, or L158.
- 1 12. The reverse transcriptase of claim 1, wherein the mutation in the processivity domain
2 comprises one or more of the following mutations corresponding to the amino acids in MMLV-
3 RT: H638G, Y586A, D653N, D524N, D524E and E562D and the mutation in the nucleotide
4 selection domain comprises F155Y.
- 1 13. The reverse transcriptase of claim 1, wherein the reverse transcriptase produces a yield of
2 greater than about 1 ug of an aRNA from 100 ng of template RNA in a single amplification
3 reaction.
- 1 14. The reverse transcriptase of claim 1, wherein the reverse transcriptase produces a yield of
2 greater than about 5 ug of an aRNA from 100 ng of template RNA in a single amplification
3 reaction.
- 1 15. The reverse transcriptase of claim 1, wherein the reverse transcriptase produces a yield of
2 greater than about 7 ug of an aRNA from 100 ng of template RNA in a single amplification
3 reaction.
- 1 16. The reverse transcriptase of claim 1, wherein the reverse transcriptase produces a yield of
2 greater than about 10 ug of an aRNA from 100 ng of template RNA in a single amplification
3 reaction.
- 1 17. The reverse transcriptase of claim 1, wherein the reverse transcriptase produces a yield of
2 greater than about 15 ug of an aRNA from 100 ng of template RNA in a single amplification
3 reaction.
- 1 18. The reverse transcriptase of claim 1, wherein the reverse transcriptase produces a yield of
2 greater than about 25 ug of an aRNA from 100 ng of template RNA in a single amplification
3 reaction.
- 1 19. The reverse transcriptase of claim 1, wherein the reverse transcriptase produces a yield of
2 greater than about 1 ug of an aRNA from 10 pg of template RNA after a two-round amplification
3 reaction.

- 1 20. The reverse transcriptase of claim 1, wherein the reverse transcriptase produces a yield of
2 greater than about 2 ug of an aRNA from 10 pg of template RNA after a two-round amplification
3 reaction.
- 1 21. The reverse transcriptase of claim 1, wherein the reverse transcriptase produces a yield of
2 greater than about 5 ug of an aRNA from 10 pg of template RNA after a two-round amplification
3 reaction.
- 1 22. The reverse transcriptase of claim 1, wherein the reverse transcriptase produces a yield of
2 greater than about 10 ug of an aRNA from 10 pg of template RNA after a two-round
3 amplification reaction.
- 1 23. The reverse transcriptase of claim 1, wherein the reverse transcriptase produces a cDNA
2 greater than about 6, 9 or even 11 kilobases in a single cDNA synthesis reaction.
- 1 24. The reverse transcriptase of claim 1, wherein the reverse transcriptase produces a cDNA
2 greater than about 6 to about 15 kilobases in a single cDNA synthesis reaction.
- 1 25. The reverse transcriptase of claim 1, wherein the reverse transcriptase produces a cDNA
2 greater than about 15 kilobases in a single cDNA synthesis reaction.
- 1 26. The reverse transcriptase of claim 1, wherein the DNA polymerase activity is greater than
2 about 200 Units per microgram.
- 1 27. The reverse transcriptase of claim 1, wherein the DNA polymerase activity is between
2 about 0.1 and 300 Units per microgram.
- 1 28. The reverse transcriptase of claim 1, wherein the RNase H activity is between about 0.1
2 and about 25 percent of the wild-type RNase H activity.
- 1 29. An isolated reverse transcriptase having substantially reduced RNase H activity
2 comprising one or more point mutations in the processivity domain.
- 1 30. The reverse transcriptase of claim 29, wherein the RNase H activity is between about 0.1
2 and 50 % of wild-type activity.
- 1 31. The reverse transcriptase of claim 29, wherein the RNase H activity is between about 1
2 and 10 % of wild-type activity.

- 1 32. The reverse transcriptase of claim 29, wherein the mutation in the processivity domain
2 comprises one or more of mutations in the following residues in MMLV-RT: H638, Y586, D653,
3 D524, D524 and E562.
- 1 33. The reverse transcriptase of claim 29, wherein the mutation in the processivity domain
2 comprises one or more of the following mutations corresponding to the amino acids in MMLV-
3 RT: H638G, Y586A, D653N, D524N, D524E and E562D.
- 1 34. The reverse transcriptase of claim 29, further comprising a mutation in the nucleotide
2 selection domain comprises a mutation of residue F155 in MMLV-RT.
- 1 35. The reverse transcriptase of claim 29, wherein the mutation in the processivity domain
2 comprises one or more of the following mutations corresponding to the amino acids in MMLV-
3 RT: H638G, Y586A, D653N, D524N, D524E and E562D and further comprising a mutation in
4 the nucleotide selection domain such as F155Y.
- 1 36. An isolated protein comprising DNA polymerase activity and substantially reduced
2 RNase H activity comprising one or more mutations in the processivity domain and the
3 nucleotide selection domain.
- 1 37. The protein of claim 36, wherein the protein produces a yield of greater than about 1, 5,
2 7, 10, 12, 15, 25 ug of an aRNA from 100 ng of template RNA in a single amplification reaction.
- 1 38. The protein of claim 36, wherein the protein produces an aRNA yield of greater than
2 about 20% as compared to an equivalent wild-type Reverse Transcriptase enzyme.
- 1 39. The protein of claim 36, wherein the protein produces a yield of greater than about 5 or
2 10 ug of an aRNA from 10 pg of template RNA after a two-round amplification reaction.
- 1 40. The protein of claim 36, wherein the protein produces a cDNA greater than about 6, 9 or
2 even 11 kilobases in a single cDNA synthesis reaction.
- 1 41. The protein of claim 36, wherein the protein produces a cDNA greater than about 6 to
2 about 15 kilobases in a single cDNA synthesis reaction.
- 1 42. The protein of claim 36, wherein the protein produces a cDNA greater than about 15
2 kilobases in a single cDNA synthesis reaction.

- 1 43. The protein of claim 36, wherein the DNA polymerase activity is greater than about 200
2 Units per microgram.
- 1 44. The protein of claim 36, wherein the DNA polymerase activity is between about 0.1 and
2 300 Units per microgram.
- 1 45. The protein of claim 36, wherein the RNase H activity is between about 0.1 and about 25
2 percent of the wild-type RNase H activity.
- 1 46. An isolated and purified reverse transcriptase protein comprising one or more mutations
2 in the nucleotide selection domain.
- 1 47. The reverse transcriptase of claim 46, wherein the reverse transcriptase is selected from
2 the group consisting of AMV, M-MLV, HTLV-1, BLV, RSV, HFV, R2 *Bombyx mori*, and HIV
3 reverse transcriptase.
- 1 48. The reverse transcriptase of claim 46, wherein the reverse transcriptase further comprises
2 a modified nucleotide sequence that encodes a modified amino acid sequence in the processivity
3 domain corresponding to amino acids 497 to 671 of M-MLV reverse transcriptase.
- 1 49. The reverse transcriptase of claim 46, further comprising one point mutation in the
2 nucleotide selection domain corresponding to amino acids 153 to 158 of MMLV reverse
3 transcriptase.
- 1 50. The reverse transcriptase of claim 46, wherein the reverse transcriptase may be used in
2 the preparation of full-length cDNA.
- 1 51. The reverse transcriptase of claim 46, wherein the mutation in the processivity domain
2 comprises one or more of the following mutations corresponding to the amino acids in MMLV-
3 RT: H638G, Y586A, D653N, D524N, D524E and E562D.
- 1 52. The reverse transcriptase of claim 46, wherein the reverse transcriptase produces a yield
2 of greater than about 1, 5, 7, 10, 12, 15, 25, 40 or 50 ug of an aRNA from 100 ng of template
3 RNA in a single amplification reaction.

- 1 53. The reverse transcriptase of claim 46, wherein the reverse transcriptase produces a yield
2 of greater than about 1, 5 or 10 ug of an aRNA from 10 pg of template RNA after a double
3 amplification reaction.
- 1 54. The reverse transcriptase of claim 46, wherein the reverse transcriptase produces a cDNA
2 greater than about 6, 9 or even 11 kilobases in a single cDNA synthesis reaction.
- 1 55. The reverse transcriptase of claim 46, wherein the reverse transcriptase produces a cDNA
2 greater than about 15 kilobases in a single cDNA synthesis reaction.
- 1 56. The reverse transcriptase of claim 46, wherein the DNA polymerase activity is greater
2 than about 200 Units per microgram.
- 1 57. The reverse transcriptase of claim 46, wherein the DNA polymerase activity is between
2 about 0.1 and 300 Units per microgram.
- 1 58. The reverse transcriptase of claim 46, wherein the RNase H activity is between about 0.1
2 and about 25 percent of the wild-type RNase H activity.
- 1 59. A reverse transcriptase protein comprising one or more mutations in the nucleotide
2 selection domain and in the processivity domain.
- 1 60. An isolated and purified protein comprising one or more mutations in the processivity
2 domain and one or more mutations in the nucleotide selection domain.
- 1 61. A process for making a protein with hyperactive reverse transcriptase activity comprising
2 the steps of:
- 3 transforming a host cell with the hyperactive reverse transcriptase comprising a mutation in the
4 processivity domain that comprises one or more of the following mutations corresponding to the
5 amino acids in MMLV-RT: H638G, Y586A, D653N, D524N, D524E and E562D and further
6 comprising a F155Y mutation in the nucleotide selection domain of MMLV-RT; and
- 7 culturing the host cell under conditions such that the hyperactive reverse transcriptase is produced
8 by the host cell.
- 1 62. An isolated and purified nucleic acid comprising a hyperactive reverse transcriptase with
2 a mutation in the processivity domain and in the nucleotide selection domain.

- 1 63. The nucleic acid of claim 62, wherein the hyperactive reverse transcriptase comprises a
2 mutation that corresponds to and includes an H638G mutation of the MMLV-RT.
- 1 64. The nucleic acid of claim 62, wherein the hyperactive reverse transcriptase comprises a
2 hyperactive reverse transcriptase further comprising an F155Y mutation.
- 1 65. The nucleic acid of claim 62, wherein the hyperactive reverse transcriptase comprises a
2 hyperactive reverse transcriptase further comprising an F155Y mutation and an H638G mutation.
- 1 66. The nucleic acid of claim 62, wherein the nucleic acid of SEQ ID No.: 1 further
2 comprises a nucleic acid segment encoding a leader sequence.
- 1 67. The nucleic acid of claim 62, wherein the nucleic acid of SEQ ID NO.: 1 further
2 comprises a nucleic acid segment encoding a protein segment other than the hyperactive reverse
3 transcriptase.
- 1 68. An isolated and purified nucleic acid that encodes a hyperactive reverse transcriptase
2 comprising one or more mutations in the processivity domain.
- 1 69. The reverse transcriptase of claim 68, wherein the mutation in the processivity domain
2 comprises one or more of mutations in the following residues in MMLV-RT: H638, Y586, D653,
3 D524, D524 and E562.
- 1 70. The nucleic acid of claim 68, wherein the hyperactive reverse transcriptase comprises an
2 H638G mutation.
- 1 71. The nucleic acid of claim 68, wherein the hyperactive reverse transcriptase comprises a
2 hyperactive reverse transcriptase further comprising an F155Y mutation and an H638G mutation.
- 1 72. The nucleic acid of claim 68, wherein the nucleic acid of SEQ ID NO.: 1 further
2 comprises a nucleic acid segment encoding a leader sequence.
- 1 73. The nucleic acid of claim 68, wherein the nucleic acid of SEQ ID NO.: 1 further
2 comprises a nucleic acid segment encoding a protein segment other than the hyperactive reverse
3 transcriptase.

- 1 74. A vector comprising a nucleic acid that comprises a nucleic acid encoding a hyperactive
2 reverse transcriptase comprising a mutation in the processivity domain and in the nucleotide
3 selection domain.
- 1 75. A vector comprising a nucleic acid that comprises a nucleic acid encoding a hyperactive
2 reverse transcriptase comprising a mutation in the nucleotide selection domain.
- 1 76. A vector comprising a nucleic acid that comprises a nucleic acid encoding a hyperactive
2 reverse transcriptase comprising a mutation in the processivity domain.
- 1 77. A host cell transformed with an expression vector comprising a nucleic acid encoding an
2 amino acid of SEQ ID NO.: 2 that encodes a hyperactive reverse transcriptase.
- 1 78. The host cell of claim 77, wherein the host cell comprises E. coli.
- 1 79. A host cell transformed to express a hyperactive reverse transcriptase.
- 1 80. A process for making an isolated hyperactive reverse transcriptase comprising the steps
2 of:
3 transforming a host cell with an isolated nucleic acid that encodes a hyperactive reverse
4 transcriptase; and
5 culturing the host cell under conditions such that the hyperactive reverse transcriptase is
6 produced.
- 1 81. The process of claim 80, wherein the hyperactive reverse transcriptase comprises an
2 H638G mutation.
- 1 82. The process of claim 80, wherein the hyperactive reverse transcriptase comprises a
2 hyperactive reverse transcriptase further comprising an F155Y mutation.
- 1 83. The process of claim 80, wherein the hyperactive reverse transcriptase comprises a
2 hyperactive reverse transcriptase further comprising an F155Y;H638G mutation.
- 1 84. A hyperactive reverse transcriptase in which one or more mutations replace at least one
2 of the amino acids of the processivity domain and the nucleotide selection domain, with an
3 alternative naturally occurring L-amino acid, the replacement being selected from the group

4 consisting of: (1) a substitution of any of isoleucine, valine, and leucine for any other of these
5 amino acids; (2) a substitution of aspartic acid for glutamic acid or vice versa; (3) a substitution
6 of glutamine for asparagine or vice versa; (4) a substitution of serine for threonine or vice versa;
7 (5) a substitution of glycine for alanine or vice versa; (6) a substitution of alanine for valine or
8 vice versa; (7) a substitution of methionine for any of leucine, isoleucine, or valine and vice
9 versa; and (8) a substitution of lysine for arginine or vice versa.

1 85. The reverse transcriptase of claim 84, wherein the replacement is selected from the group
2 consisting of: (1) a substitution of any of isoleucine, valine, or leucine for any other of these
3 amino acids; (2) a substitution of aspartic acid for glutamic acid or vice versa; (3) a substitution
4 of glutamine for asparagine or vice versa; and (4) a substitution of serine for threonine or vice
5 versa and wherein the hyperactive reverse transcriptase comprises a hyperactive reverse
6 transcriptase.

1 86. A kit for nucleic acid synthesis, comprising, in a suitable container:

2 a hyperactive reverse transcriptase; and

3 a reaction solution for the reverse transcriptase.

1 87. The kit of claim 86, further comprising an insert that comprises information for using the
2 reverse transcriptase.

1 88. The kit of claim 86, wherein the reaction solution comprises a reverse transcriptase
2 reaction buffer.

1 89. The kit of claim 86, further comprising a primer.

1 90. The kit of claim 86, wherein the reaction solution comprises a reverse transcriptase
2 buffer.

1 91. The kit of claim 86, wherein the reaction solution comprises a PCR buffer.

1 92. The kit of claim 86, further comprising a mix of nucleotides.

1 93. The kit of claim 86, further comprising containers comprising individual nucleotides.

- 1 94. The kit of claim 86, wherein the reaction solution comprises a buffer for in vitro
2 transcription.
- 1 95. The kit of claim 86, further comprising a template purification column.
- 1 96. The kit of claim 86, further comprising magnetic particles suitable for nucleic acid
2 purification.
- 1 97. A kit for nucleic acid synthesis, comprising, in a suitable container:
2 a hyperactive reverse transcriptase comprising one point mutation in the processivity domain; and
3 a reaction solution for the reverse transcriptase.
- 1 98. A kit for nucleic acid synthesis, comprising, in a suitable container:
2 a hyperactive reverse transcriptase comprising one point mutation in the processivity domain and
3 one point mutation in the nucleotide selection domain; and
4 a reaction solution for the reverse transcriptase.
- 1 99. A method for RNA amplification comprising the steps of:
2 reverse transcribing an RNA template into a single-stranded cDNA with a hyperactive reverse
3 transcriptase in the presence of an oligonucleotide comprising a transcriptional promoter and a
4 primer;
5 purifying the single-stranded cDNA; and
6 generating amplified RNA (aRNA) using an RNA polymerase.
- 1 100. A method for RNA amplification comprising the steps of:
2 reverse transcribing an RNA template into a single-stranded cDNA with a hyperactive reverse
3 transcriptase in the presence of an oligonucleotide comprising a transcriptional promoter and a
4 primer;
5 converting the single-stranded cDNA into double-stranded cDNA using a DNA polymerase;
6 purifying the double-stranded cDNA; and

- 7 generating amplified RNA (aRNA) using an RNA polymerase.
- 1 101. The method of claim 100, further comprising the step of purifying the aRNA.
- 1 102. A kit for RNA amplification, comprising, in a suitable container:
- 2 a hyperactive reverse transcriptase comprising one or more point mutations in the processivity
3 domain and one or more point mutations in the nucleotide selection domain; an oligonucleotide
4 comprising a transcriptional promoter region and oligo(dT) region; a DNA polymerase; and an
5 RNA polymerase.
- 1 103. The kit of claim 102, further comprising an insert that comprises information for using
2 the optimized reverse transcriptase.
- 1 104. The kit of claim 102, wherein the reaction solution comprises a 10X concentrated reverse
2 transcriptase reaction buffer.
- 1 105. The kit of claim 102, further comprising a primer.
- 1 106. The kit of claim 102, wherein the reaction solution comprises a reverse transcriptase
2 buffer.
- 1 107. The kit of claim 102, wherein the reaction solution comprises a DNA Polymerase buffer.
- 1 108. The kit of claim 102, further comprising a mix of nucleotides.
- 1 109. The kit of claim 102, further comprising containers comprising individual nucleotides.
- 1 110. The kit of claim 102, wherein the reaction solution comprises a buffer for in vitro
2 transcription.
- 1 111. The kit of claim 102, further comprising a nucleic acid purification column.
- 1 112. The kit of claim 102, further comprising a magnetic particle or particles suitable for
2 nucleic acid purification.
- 1 113. An aRNA made by the method of claim 995.
- 1 114. An aRNA made by the method of claim 100.

- 1 115. A kit for RNA amplification, comprising, in a suitable container:
2 a hyperactive reverse transcriptase comprising one or more point mutations in the processivity
3 domain; an oligonucleotide comprising a transcriptional promoter region and oligo(dT) region; a
4 DNA polymerase; and an RNA polymerase.
- 1 116. The kit of claim 115, further comprising an insert that comprises information for using
2 the optimized reverse transcriptase.
- 1 117. The kit of claim 115, wherein the reaction solution comprises a 10X concentrated reverse
2 transcriptase reaction buffer.
- 1 118. The kit of claim 115, further comprising a primer.
- 1 119. The kit of claim 115, wherein the reaction solution comprises a reverse transcriptase
2 buffer.
- 1 120. The kit of claim 115, wherein the reaction solution comprises a DNA polymerase buffer.
- 1 121. The kit of claim 115, further comprising a mix of nucleotides.
- 1 122. The kit of claim 115, further comprising containers comprising individual nucleotides.
- 1 123. The kit of claim 115, wherein the reaction solution comprises a buffer for in vitro
2 transcription.
- 1 123. The kit of claim 115, further comprising a nucleic acid purification column.
- 1 125. The kit of claim 115, further comprising one or more magnetic particles suitable for
2 nucleic acid purification.
- 1 126. An aRNA comprising:
2 a ssDNA or a DNA:RNA hybrid made from an RNA template by a hyperactive reverse
3 transcriptase.
- 1 127. An RT-PCR kit comprising in one or more suitable containers: a hyperactive reverse
2 transcriptase, two or more primers, nucleotides, a thermostable DNA polymerase and an RT-PCT
3 buffer.

- 1 128. The RT-PCR kit of claim 127, wherein the container comprising a hyperactive reverse
- 2 transcriptase further comprises one or more reverse transcriptases.